Xenopus differentiation: **VegT gets specific**

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Depletion of the maternal store of the localised mRNA encoding the T-box transcription factor VegT in *Xenopus* embryos has recently been shown to dramatically block endoderm formation and change the normal position of the mesodermal and ectodermal germ layers.

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Formation of the three embryonic germ layers known as endoderm, mesoderm and ectoderm is an important event during the earliest stages of development in all animals. The appearance of these germ layers marks the first outwardly discernible sign of a difference between groups of cells in an embryo and, by the end of embryogenesis, these primitive tissues will differentiate to produce the entire and diverse range of specialised organs comprising a magnificently complex organism. Endoderm will give rise to the gut and its outgrowths, which include the liver, pancreas and lungs; mesoderm will generate muscle, skeleton and blood; and ectoderm will create the skin and nervous tissue. A central question in early development is how the different identities of the germ layers first manifest themselves from a starting point of just a single cell — the egg. One mechanism believed, but not demonstrated, to be involved in this process in frogs is the asymmetric localisation of maternal determinants in the cytoplasm of the egg. Upon cell cleavage, a different developmental potential is then believed to be conferred upon daughter cells according to the amount of the cytoplasmic determinant that they receive.

Now, Zhang *et al.* [1] have provided striking evidence that a mechanism involving asymmetric localisation is indeed operating in *Xenopus*. By depleting the maternal store of a localised mRNA encoding a T-box transcription factor called VegT [2] (also known as Antipodean, Xombi and Brat; references within [1]), endoderm formation was blocked and the correct pattern of germ layer specification in the blastula was subsequently disrupted. For the first time, this study definitively demonstrates that an asymmetrically expressed maternal mRNA is crucial for the specification of a germ layer in a vertebrate. Furthermore, Tada *et al.* [3] have now identified a gene called *Bix1* which is possibly directly induced by VegT and may be responsible for mediating the effects of VegT in the mesoderm and endoderm. Together, these two recent reports may enable us to start piecing together the pathway that directs the very earliest stages of germ layer specification in the *Xenopus* embryo.

Signalling molecules in endoderm and mesoderm formation in *Xenopus*

Our understanding of germ layer specification in the *Xenopus* embryo is mostly restricted to that of the mesoderm. Comparatively little is known about the endoderm due to a lack, until just recently, of endoderm-specific gene markers. However, signalling by transforming growth factor β (TGFβ) type molecules, a family of secreted growth factors involved with inductive signals during embryonic development, is thought to be important for the formation of both germ layers. In the case of mesoderm, a secreted factor arising from the presumptive endodermal cells of the vegetal pole is believed to induce neighbouring equatorial cells to adopt a mesodermal fate (reviewed in [4]). Nieuwkoop demonstrated this principle many years ago by showing that a tissue explant from the vegetal pole could induce an overlying animal pole explant, normally fated to become ectoderm, to form mesoderm instead (Figure 1). The identity of the signalling molecule responsible for this process in the frog has been long sought after and, although it is still unknown, it appears likely to be a member of the TGFβ family.

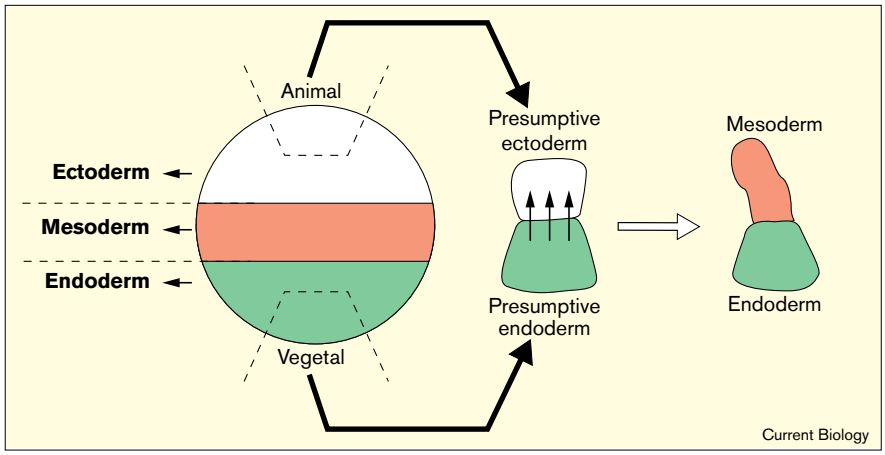
The involvement of similar signalling processes in endoderm formation has been demonstrated by the ability of some TGFβ family molecules to induce both mesoderm and endoderm when ectopically expressed in undifferentiated tissue [5]. Moreover, endoderm formation in the embryo is disrupted following expression of a 'dominant-negative' receptor that blocks signalling by the TGFβ molecule activin [5], or following expression of mutant versions of Vg1, another TGFβ family member [6].

The role of VegT

Zhang *et al.* [1] have now demonstrated unequivocally that the maternally provided T-box transcriptional activator VegT is essential for endoderm formation. All members of the T-box transcription factor family, like the prototypic member Brachyury, are implicated as critical regulators of early embryonic differentiation [7]. *VegT* mRNA is expressed in both the early mesoderm and the endoderm and, when misexpressed, can ectopically induce endodermal [8] and mesodermal marker genes ([2,8] and references in [1]). *VegT* is unique among the family members so far identified, however, in that its mRNA is maternally expressed at a high level and these transcripts are strictly localised to the vegetal pole of oocytes. Ever since its discovery, the significance of this localisation and the role of

Figure 1

In a simplified fate map of the *Xenopus* blastula, endoderm, mesoderm and ectoderm are generated from the vegetal, equatorial and animal pole cells respectively. Vegetal pole explants have been shown to secrete a factor that induces mesoderm formation in animal pole explants which are normally fated to form ectoderm.



VegT in mesoderm and endoderm formation have been an enticing mystery.

Zhang's approach to unravel the function of VegT was to degrade the maternal content of *VegT* mRNA. Injection of antisense oligonucleotides that are complementary to sequences in the mRNA can cause specific destruction of the maternal mRNA in the fully grown oocyte by the action of endogenous RNase H. Implantation of these oocytes back into the frog and subsequent fertilisation revealed the fate of embryos containing drastically depleted maternal *VegT* mRNA levels. These embryos developed normally up until the start of gastrulation but then exhibited striking abnormalities. Importantly, there was a complete lack of endoderm formation as judged by an absence of endoderm-specific gene expression. This demonstrated that maternal *VegT* is indeed a maternal determinant essential for endoderm specification. Another major effect was that vegetal pole explants had lost their ability to induce mesoderm in overlying presumptive ectodermal explants (in repeats of the experiment shown in Figure 1), indicating that maternal *VegT* is also crucial for the release of the currently unknown, secreted mesoderm-inducing factor.

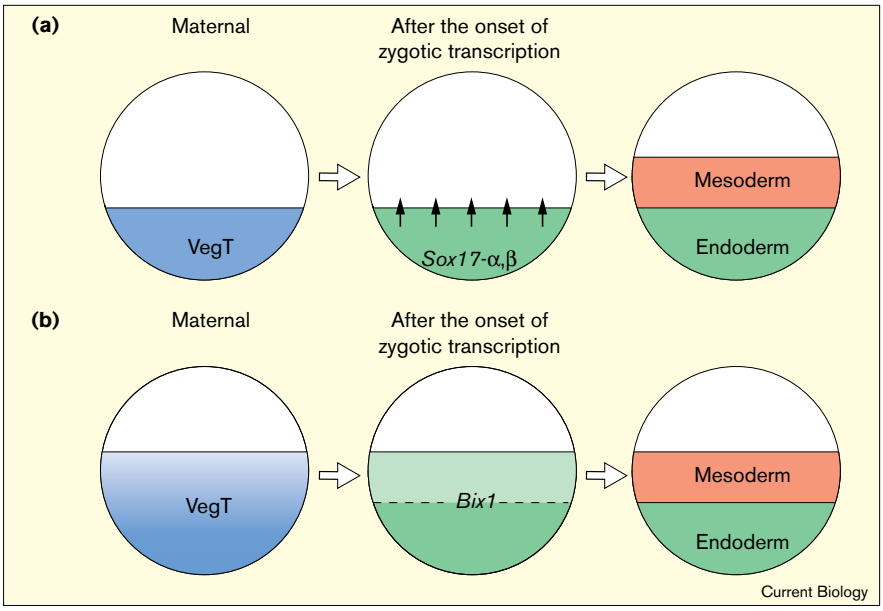
Intriguingly, embryos depleted of maternal *VegT* also showed a vegetal shift in the positioning of the remaining germ layers such that mesoderm and ectoderm replaced endoderm in the vegetal pole. This phenotype is similar to, but more extensive than, that obtained when TGFβ signalling is blocked in the vegetal pole, whereby mesoderm forms in cells normally fated to form endoderm [5]. This finding possibly provides further evidence that VegT acts upstream of a TGFβ family molecule and that, in addition to being important for mesoderm formation, VegT is also required for endoderm formation. The reason for the formation of mesoderm in the vegetal pole of *VegT*-depleted embryos is unclear, but one possibility is that maternal *VegT* may synergise with an early, maternally provided TGFβ signalling molecule. We already know of a potential candidate in the form of the vegetally localised growth factor, Vg1 [6,9]. The combined action of VegT and growth factors such as Vg1 may therefore be necessary for endoderm formation in the vegetal pole: if either component is lacking, mesoderm forms instead of endoderm.

Downstream targets of VegT

The absence of endodermal markers in *VegT*-depleted embryos indicated that VegT is likely to be activating genes that specify an endodermal fate at the onset of zygotic transcription. Potential candidates are the transcription factors *Sox17-α* and *Sox17-β* [10] and *Mixer* [11], because their expression commences after the onset of zygotic transcription, they are restricted to the endoderm, and they have been shown to mediate an endodermal fate in explants. Indeed, VegT can activate *Sox17-β* expression in animal pole explants (A. Zorn and F.S., unpublished observations). Thus, one model of early differentiation may involve the induction of endoderm by maternal VegT with the subsequent induction of mesoderm in the overlying equatorial region (Figure 2a).

Tada *et al.* [3] have now reported the identification of a VegT-binding site in the promoter of a gene encoding the paired-box homeobox transcription factor Bix4 and have thus pinpointed a gene that is likely to be directly induced by VegT. Indeed, they have shown that VegT can induce the expression of a highly related gene, *Bix1*, in an animal cap assay. In contrast to the genes *Mixer*, *Sox17-α* and *Sox17-β* which are endoderm specific [10,11], *Bix1* mRNA is expressed in both the endoderm and mesoderm of the embryo, with higher levels being observed in the vegetal pole of the early gastrula [3]. Maternal VegT may therefore be required in both these germ layers for induction of *Bix1*. Interestingly, Bix1 can induce endoderm and mesoderm in a dose-dependent manner: if *Bix1* is misexpressed in presumptive ectoderm at high concentrations it induces

Figure 2



Two potential models for the role of VegT in endoderm and mesoderm specification. **(a)** Maternal *VegT* mRNA is translated in the presumptive endoderm of the vegetal pole and activates endoderm-specific genes such as *Sox17-α* and *Sox17-β* at the onset of zygotic transcription. VegT is also responsible for the activity of a secreted molecule that induces mesoderm in an equatorial position and that may also help specify the endoderm in VegT-expressing cells. **(b)** Maternal *VegT* mRNA is translated in the endoderm and mesoderm, potentially in a vegetal to animal gradient. At the onset of zygotic transcription, it activates genes such as *Bix1* in both presumptive germ layers and potentially at high levels in the vegetal pole and lower levels in the equatorial region. In this way, VegT may mediate – in a concentration-dependent manner – the formation of endoderm and mesoderm, respectively, in these regions of the embryo.

endoderm, but if it is expressed at lower concentrations it induces ventral mesoderm [3].

This leads to another speculative model in which maternal VegT may be required in both the vegetal pole and the equatorial region to induce genes such as *Bix1* at higher concentrations in the vegetal pole and at lower concentrations in the equatorial region, thereby generating endoderm in vegetal cells and mesoderm at the equator (Figure 2b). Whether a vegetal to animal gradient of VegT expression is involved in the differential expression of genes in the different germ layers remains to be established. The real situation will almost certainly be more complex because *Bix1* is also induced in an immediate-early fashion by activin [3], another potential maternal signalling molecule (reviewed in [4]); perhaps this is again an indication of potential co-ordinate regulation of downstream genes by VegT and maternal TGFβ-like signalling molecules.

This discussion represents a necessary simplification of some, but not all, potential models for maternal VegT function, and I urge readers to refer to the paper by Zhang *et al.* in *Cell* [1] for an excellent description of their models for the role of this transcription factor in germ layer specification. Identifying which model is the most likely reflection of mesoderm and endoderm specification in the embryo will require more information than we have at the moment. We still need to understand more about the relationship between VegT and maternally provided signalling molecules. Furthermore, we still do not know the distribution of VegT protein after it is translated from the maternal store of mRNA — neither where it is expressed with respect to the different germ layers, nor whether it forms a gradient that might activate genes in a concentration-dependent manner. The answers may bring us closer to understanding the earliest developmental decisions that underlie the formation of both these fundamental germ layers.

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